IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
INTERNATIONAL PRELIMINARY EXAMINATION
AUTHORITY (IPEA/US)

INTERNATIONAL APPLICATION NO.: PCT/US03/09428
INTERNATIONAL FILING DATE: 27 March 2003
APPLICANT: MEDICAL COLLEGE OF OHIO
TITLE: METHOD AND COMPOSITIONS FOR THE DIAGNOSIS AND TREATMENT OF NON-SMALL CELL LUNG CANCER USING GENE EXPRESSION PROFILES

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

October 2003

STATEMENT UNDER ARTICLE 34
ACCOMPANYING AMENDMENT

Sir:

The invention relates to solid phase hybridization templates for measuring, in a standardized fashion, PCR products following standardized quantitative reverse transcription-PCR process, as reflected in amended claims 58-66.

The invention also relates to a method for preparing a solid base hybridization templates for measuring, in the standardized fashion, PCR products following standardized quantitative reverse-transcription PCR, as now reflected in new claims 81-93.

The amended and new claims do not go beyond the disclosure of the International application as filed; but rather, further define the present invention.

Respectfully submitted,

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LETTER ACCOMPANYING AMENDMENT
UNDER ARTICLE 34

Sir:

Enclosed please find Substitute Sheets 71-84/3 amending the claim as indicated below:

Claims 1-57 -- Unchanged

Claims 58-64 – Amended

Claims 65-80 – Unchanged

Claims 81-93 – Added

Applicant requests that the amendments under Article 34 to the claims be taken into account by the International Preliminary Examining Authority.

Respectfully submitted,

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CLAIMS

We claim:

1. A method for determining whether an agent can be used to reduce the proliferation and/or cause the death of cancer cells or inhibit the growth of a cancer cell population, comprising the steps of: a) obtaining a sample of cancer cells; b) determining and quantifying the level of expression in the cancer cells of a marker identified in Tables 1 and 5; and c) identifying that an agent can be used to reduce the proliferation and/or cause the death of said cancer cells when the marker is expressed at a certain level.

2. The method of claim 1, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or portion thereof, wherein the transcribed polynucleotide comprises the marker.

3. The method of claim 2, wherein the transcribed polynucleotide is an mRNA or siRNA.

4. A method of claim 2, wherein the transcribed polynucleotide is cDNA.

5. The method of claim 1, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a protein or protein fragment corresponding to the marker.

6. The method of claim 2, wherein the step of detecting further comprises amplifying the transcribed polynucleotide using RT-PCR.

7. The method of claim 6, wherein the step of detecting the level of expression includes:

initially screening a plurality of genes representing different functional classes,

evaluating an expanded group of genes represented by genes that are positively or negatively associated in the initial screening,

comparing the expression of the positively and negatively associated genes to form at least one interactive gene expression index (IGEI),

using individual gene analysis and IGEI analysis, and

developing at least one model of the level that describes an association between the level of detection of the expression of at least one of the markers identified in Tables 1 and 5 and reduced proliferation and/or increased death.

8. The method of claim 5, wherein the presence of the protein or protein fragment is detected using a reagent which specifically binds with the protein or protein fragment.

9. The method of claim 8, wherein the reagent is selected from the group consisting of an antibody, an antibody derivative, and an antibody fragment.

10. The method of claim 1, wherein the cancer cells are selected from the group consisting of cancer cell lines and cancer cells obtained from a patient.

11. The method of claim 1, wherein the agent is a chemotherapeutic compound.

12. The method of claim 11, wherein the agent is a platinum compound.

13. The method of claim 12, wherein the agent is cisplatin.

14. A method for determining whether an agent is effective in treating cancer, comprising the steps of: a) obtaining a sample of cancer cells; b) exposing the sample to an agent; c) determining and quantifying the level of expression of a marker identified in Tables 1 and 5 in the sample exposed to the agent and in a sample that is not exposed to the agent; and d) identifying that an agent is effective in treating cancer when expression of the marker is altered in the presence of said agent.

15. The method of claim 14, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or portion thereof, wherein the transcribed polynucleotide comprises the marker.

16. The method of claim 15, wherein the transcribed polynucleotide is an mRNA or siRNA.

17. A method of claim 15, wherein the transcribed polynucleotide is cDNA.

18. The method of claim 14, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a protein or protein fragment corresponding to the marker.

19. The method of claim 15, wherein the step of detecting further comprises amplifying the transcribed polynucleotide using RT-PCR.

20. The method of claim 19, wherein the step of detecting the level of expression includes:

initially screening a plurality of genes representing different functional classes,

evaluating an expanded group of genes represented by genes that are positively associated in the initial screening,

comparing the expression of the positively or negatively associated genes to form at least one interactive gene expression index (IGEI),

using the IGEI analysis, and

developing at least one model that describes an association between the level of detection of the expression of at least one of the markers identified in Tables 1 and 5 and reduced proliferation and/or cell death.

21. The method of claim 19, wherein the presence of the protein or protein fragment is detected using a reagent which specifically binds with the protein or protein fragment.

22. The method of claim 21, wherein the reagent is selected from the group consisting of an antibody, an antibody derivative, and an antibody fragment.

23. The method of claim 14, wherein the cancer cells are selected from the group consisting of cancer cell lines and cancer cells obtained from a patient.

24. The method of claim 14, wherein the agent is a chemotherapeutic compound.

25. The method of claim 24, wherein the agent is a platinum compound.

26. The method of claim 26, wherein the agent is cisplatin.

27. A method for determining whether treatment with an agent should be continued in a cancer patient, comprising the steps of: a) obtaining two or more samples comprising cancer cells from a patient during the course of treatment with the agent; b) determining and quantifying the level of expression of a marker identified in Tables 1 and 5 in the two or more samples; and c) continuing treatment when the expression level of the marker is not significantly altered during the course of treatment.

28. The method of claim 27, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or portion thereof, wherein the transcribed polynucleotide comprises the marker.

29. The method of claim 28, wherein the transcribed polynucleotide is an mRNA or siRNA.

30. A method of claim 28, wherein the transcribed polynucleotide is cDNA.

31. The method of claim 27, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a protein or protein fragment corresponding to the marker.

32. The method of claim 28, wherein the step of detecting further comprises amplifying the transcribed polynucleotide using RT-PCR.

33. The method of claim 32, wherein the step of detecting the level of expression includes:

initially screening a plurality of genes representing different functional classes,

evaluating an expanded group of genes represented by genes that are positively or negatively associated in the initial screening,

comparing the expression of the positively and negatively associated genes to form at least one interactive gene expression index (IGEI),

using the IGEI analysis, and

developing at least one model that describes an association between the level of detection of the expression of at least one of the markers identified in Tables 1 and 5 and reduced proliferation and/or cell death.

34. The method of claim 31, wherein the presence of the protein or protein fragment is detected using a reagent which specifically binds with the protein or protein fragment.

35. The method of claim 34, wherein the reagent is selected from the group consisting of an antibody, an antibody derivative, and an antibody fragment.

36. The method of claim 27, wherein the cancer cells are selected from the group consisting of cancer cell lines and cancer cells obtained from a patient.

37. The method of claim 29, wherein the agent is a chemotherapeutic compound.

38. The method of claim 38, wherein the agent is a platinum compound.

39. The method of claim 38, wherein the agent is cisplatin.

40. A method for identifying new cancer treatments, comprising the steps of: a) obtaining a sample of cancer cells; b) determining and quantifying the level of expression of a marker identified in Tables 1 and 5; c) exposing the sample to the cancer treatment; d) determining the level of expression of the marker in the sample exposed to the cancer treatment; and e) identifying that the cancer treatment is effective in treating cancer when the marker is expressed at a certain level.

41. The method of claim 40, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or portion thereof, wherein the transcribed polynucleotide comprises the marker.

42. The method of claim 41, wherein the transcribed polynucleotide is an mRNA or siRNA.

43. A method of claim 41, wherein the transcribed polynucleotide is cDNA.

44. The method of claim 40, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a protein or protein fragment corresponding to the marker.

45. The method of claim 40, wherein the step of detecting further comprises amplifying the transcribed polynucleotide using RT-PCR.

46. The method of claim 45, wherein the step of detecting the level of expression includes:

initially screening a plurality of genes representing different functional classes,

evaluating an expanded group of genes represented by genes that are positively or negatively associated in the initial screening,

comparing the expression of the positively and negatively associated genes to form at least one interactive gene expression index (IGEI),

using the IGEI analysis, and

developing at least one model that describes an association between the level of detection of the expression of at least one of the markers identified in Tables 1 and 5 and reduced proliferation and/or cell death.

47. The method of claim 44, wherein the presence of the protein or protein fragment is detected using a reagent which specifically binds with the protein or protein fragment.

48. The method of claim 47, wherein the reagent is selected from the group consisting of an antibody, an antibody derivative, and an antibody fragment.

49. The method of claim 40, wherein the cancer cells are selected from the group consisting of cancer cell lines and cancer cells obtained from a patient.

50. The method of claim 40, wherein the agent is a chemotherapeutic compound.

51. The method of claim 50, wherein the agent is a platinum compound.

52. The method of claim 51, wherein the agent is cisplatin.

53. A method of diagnosing non small cell lung cancer in a patient, comprising: (a) detecting and quantifying the level of expression in a tissue sample of c-myc, E2F-1 and p21 genes; wherein differential expression of the c-myc, E2F-1 and p21 genes is indicative of non small cell lung cancer.

54. A method of detecting the progression of non small cell lung cancer in a patient, comprising: (a) detecting and quantifying the level of expression in a tissue sample of two or more c-myc, E2F-1 and p21 genes; wherein differential expression of the c-myc, E2F-1 and p21 genes is indicative of non small cell lung cancer progression.

55. A method of monitoring the treatment of a patient with non small cell lung cancer, comprising: (a) administering a pharmaceutical

composition to the patient; (b) preparing a gene expression profile from a cell or tissue sample from the patient; and (c) comparing the patient gene expression profile to a gene expression from a cell population selected from the group consisting of normal lung cells; and non small cell lung cancer.

56. A method of treating a patient with non small cell lung cancer, comprising: (a) administering to the patient a pharmaceutical composition, wherein the composition alters the expression of at least one gene in Tables 1 and 5 or c-myc, E2F-1 and p21 genes; (b) preparing an IGEI comprising standardized gene expression values using StaRT-PCR from a cell or tissue sample comprising tumor cells obtained before treatment and another sample obtained after treatment; and (c) comparing the sample obtained prior to treatment with the sample obtained after treatment.

57. A method of screening for an agent capable of modulating the onset or progression of non small cell lung cancer, comprising: (a) preparing a first IGEI comprising standardized gene expression values using StaRT-PCR of a cell population comprising non small cell cancer cells, wherein the first IGEI determines the expression level of one or more genes from Tables 1 and 5 or c-myc, E2F-2 and p21 genes; (b) exposing the cell population to the agent; (c) preparing second IGEI comprising standardized gene expression values using StaRT-PCR of the agent-exposed cell population; and (d) comparing the first and second IGEIs.

58. A solid phase hybridization template for measuring, in a standardized fashion, PCR products following quantitative standardized RT (StaRT)-PCR amplification of at least one gene of interest comprising:

a) at least one solid phase hybridization template where, for each gene of interest, at a designated spot that is subsequently identifiable, multiple copies of an oligonucleotide of any length that will bind with 100% specificity to both a competitive template (CT) and a native template (NT) StaRT-PCR product for the gene are attached;

b) at least one first oligonucleotide which is identified such that, following labeling with fluorescent, mass specific, or other identifiable tag, the first oligonucleotide hybridizes with 100% specificity to a sequence that is present on the NT but not on the CT, which sequence comprises a region on the NT that is beyond a region homologous to the 3' end of sense strand of the CT; and,

c) at least one second oligonucleotide which is identified such that, following labeling with fluorescent, mass specific, or other identifiable tag, the second oligonucleotide hybridizes with 100% specificity to a region on the CT that spans a juncture formed when the sequence was removed from the NT to make the CT;

whereby, when CT and NT PCR products are amplified and hybridized to the designated spot on the solid phase hybridization template, each of the templates (NT and CT) represent each gene as a pair separate from templates for other genes; and,

whereby, when labeled oligonucleotide probes homologous to NT or CT are hybridized to the CT and NT PCR products, the amount of NT relative to CT PCR product bound to the spot is quantifiable.

59. The solid phase hybridization template of claim 58 wherein the NT-specific and CT-specific fluorescent-labeled oligonucleotides for multiple genes are mixed in equal amounts and hybridized to gene-specific PCR products bound to gene-specific oligonucleotides spotted on the solid phase template.

60. The solid phase hybridization template of claim 59 wherein a ratio between the amounts of each probe bound to the designated spot quantifies the NT relative to the CT.

61. The solid phase hybridization template of claim 60, wherein any difference in binding affinities between the CT and the CT probe relative to that between the NT and the NT probe is consistent between different samples assessed, and from one experiment to another.

62. The solid phase hybridization template of claim 58 wherein the template comprises at least one solid support comprising at least one of standardized microarray, microbeads, glass slides, or chips that are prepared by photolithography.

63. The solid phase hybridization template of claim 62, wherein the solid support comprises at least one of a membrane, a glass support, a filter, a tissue culture dish, a polymeric material, a bead, or a silica support.

64. The solid phase hybridization template of claim 63,wherein the solid support has adhered thereto oligonucleotides homologous to at least two genes, wherein each of the oligonucleotides comprises a sequence that specifically hybridizes to at least one gene.

65. The solid phase hybridization template of claim 64, wherein the oligonucleotides are covalently attached to the solid support.

66. The solid phase hybridization template of claim 64, wherein the oligonucleotides are non-covalently attached to the solid support.

67. A computer system comprising: (a) a database containing information identifying the standardized numerical expression level in units of molecules/10^6 β-actin molecules in lung tissue of a set of genes comprising at least two genes in Tables 1 and 5 or c0myc, E2F-1 and p21 genes; and (b) a user interface to view the information.

68. A computer system of claim 67, wherein the database further comprises sequence information for the genes.

69. A computer system of claim 67, wherein the database further comprises information identifying the standardized numerical expression level in units of molecules/10^6 β-actin molecules for the set of genes in normal lung tissue.

70. A computer system of claim 67, wherein the database further comprises information identifying the standardized numerical expression level in units of molecules/10^6 β-actin molecules of the set of genes in non small cell cancer tissue.

71. A computer system of any of claims 67-70, further comprising records including descriptive information from an external database, which information correlates said genes to records in the external database.

72. A computer system of claim 71, wherein the external database is Genbank.

73. A method of using a computer system of any one of claims 67-72 to present information identifying the standardized numerical expression level in a tissue or cell of at least one gene in Tables 1 and 5

and c-myc, E2F-1 and p21 genes, comprising: (a) comparing the standardized numerical expression level of at least one gene in Tables 1 and 5 or c-myc, E2F-1 and p21 genes in the tissue or cell to the level of expression of the gene in the database.

74. A method of claim 73, wherein the expression level of at least two genes are compared.

75. A method of claim 73, wherein the expression level of at least five genes are compared.

76. A method of claim 73, wherein the expression level of at least ten genes are compared.

77. A method of claim 73, further comprising displaying the level of expression of at least one gene in the tissue or cell sample compared to the expression level in lung cancer or in normal lung tissue.

78. A kit comprising at least one solid support of any one of claims 64-66 packaged with gene expression information for said genes.

79. A kit of claim 78, wherein the gene expression information comprises gene expression levels in a tissue or cell sample exposed to a toxin.

80. A kit of claim 79, wherein the gene expression information is in an electronic format.

81. A method for preparing a solid phase hybridization template for measuring, in a standardized fashion, PCR products of at least one

gene of interest relative to a reference gene, following standardized quantitative RT-PCR the method comprising the steps of:

a) for each gene, attaching at least one oligonucleotide of any length that binds with specificity to both a competitive template, CT, and a native template, NT, to a solid support at a designated location that is subsequently identifiable;

b) amplifying CT and NT PCR products for each gene of interest;

c) pooling the CT and NT PCR products;

d) hybridizing the CT and NT PCR products to the designated spots on the solid phase template wherein each of the templates (NT and CT) represent each gene as a pair separate from templates for other genes;

e) washing the solid phase template; and

f) preparing a first oligonucleotide probe wherein the first oligonucleotide probe is labeled with a first fluor, the first oligonucleotide probe being homologous to, and will bind to sequences unique to the NT region of the NT that are not homologous to the CT ;and

(g) preparing a second oligonucleotide probe, wherein, the second oligonucleotide probe is labeled with a second fluor which is specific to the CT, the second oligonucleotide probe being homologous to and binding to CT sequences that span the 3' end of the reverse primer; and,

(h) evaluating a region between a forward primer common to both the NT and the CT and the 3' end of the reverse CT primer.

82. The method of claim 81, wherein the NT-specific and CT-specific fluorescent-labeled oligonucleotides from steps (f) and (g) for multiple genes are mixed in equal amounts and hybridized to the gene-

specific PCR products bound to the gene-specific oligonucleotides spotted on the solid phase template.

83. The method of claim 82, wherein a ratio between the amount of each fluor bound to the designated spot quantifies the NT relative to the CT.

84. The method of claim 83, wherein, any difference in binding affinities between the CT and the CT probe relative to that between the NT and the NT probe is consistent between different samples assessed, and from one experiment to another.

85. The method of claim 81, wherein the template comprises at least one of solid support comprising at least one of standardized microarray, microbeads, glass slides, or chips that are prepared by photolithography.

86. The method of claim 85, wherein the solid support comprises at least one of a membrane, a glass support, a filter, a tissue culture dish, a polymeric material, a bead, or a silica support.

87. The method of claim 86, wherein the solid support has adhered thereto at least two oligonucleotides, wherein each of the oligonucleotides comprises a sequence that specifically hybridizes to at least one gene.

88. The method for preparing the solid phase hybridization template of claim 87, wherein the oligonucleotides are covalently attached to the solid support.

89. The method for preparing the solid phase hybridization template of claim 87, wherein the oligonucleotides are non-covalently attached to the solid support.

90. At least one molecular beacon nucleic acid having at least one region which is complementary to a nucleic acid, such that the molecular beacon is useful for quantitating the presence of the nucleic acid in a sample.

91. The molecular beacon nucleic acid of claim 90 which comprises a nucleic acid comprising a pair of complementary regions and having a fluorophore and a fluorescent quencher associated therewith.

92. The molecular beacon of claim 91 wherein the fluorophore and the fluorescent quencher are associated with different portions of the nucleic acid in such an orientation that, when the complementary regions are annealed with one another, fluorescence of the fluorophore is quenched by the fluorescent quencher.

93. The molecular beacon of claim 92 wherein, when the complementary regions of the nucleic acid are not annealed with one another, fluorescence of the fluorophore is quenched to a lesser degree.

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